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Research Article

Isotope labeled 'HEA/HEE' moiety in the synthesis of labeled HIV-protease inhibitors—Part II

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Summary

[$^2H_5$]-Amprenavir and [$^2H_5$]-saquinavir have been prepared from a common labeled precursor (1S, 2S)-(1-oxiranyl-2-[$^2H_5$]phenylethyl)-carbamic acid *tert*-butyl ester, **1**. Both of these compounds are in the 'HEA' class of HIV protease inhibitors. [$^2H_5$]-Indinavir, a representative of the 'HEE' group of protease inhibitors, has also been synthesized. In the case of indinavir, 1S-(2,2-dimethyl-8, 8a-dihydro-3aH-indeno-[1,2-d]-oxazol-3R-yl)-2-oxiranylmethyl-3-[$^2H_5$]phenylpropan-1-one, **11**, provided the [phenyl-$^2H_5$]-HEE core structure for synthesis of the desired labeled compound. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: protease inhibitor; anti-HIV; AIDS; D_5-HEA/HEE isostere

Introduction

The hydroxyethylamine (HEA) isostere in amprenavir[1] and saquinavir,[2] and the hydroxyethylene (HEE) substructure in indinavir[3] have been specifically labeled with [$^2H_5$]. Labeled versions of all three protease inhibitors were needed for analytical method development and validation.

The synthetic approach we previously employed for [$^2H_5$]-DPH 153893 and [$^2H_5$]-DPH 140662[4,5] was used to make the two hydroxyethylamine (HEA) peptide mimetics (see substructures in Figure 1), [$^2H_5$]-amprenavir and [$^2H_5$]-saquinavir. In particular, [$^2H_5$]-amprenavir and [$^2H_5$]-saquinavir were synthesized by following a sequence of reactions attaching appropriate residues to the key intermediate, (1S, 2S)-(1-oxiranyl-2-[$^2H_5$]phenylethyl)-carbamic acid *tert*-butyl ester, **1**.

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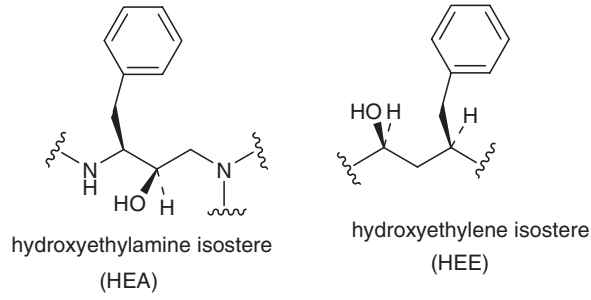


Figure 1. Substructures of HIV protease inhibitors

To make [$^2H_5$]-indinavir with deuterium similarly incorporated in the hydroxyethylene (HEE) isostere (see Figure 1) we required a different epoxide intermediate. We chose 1S-(2,2-dimethyl-8, 8a-dihydro-3aH-indeno[1,2-d]-oxazol-3R-yl)-2-oxiranylmethyl-3-[$^2H_5$]-phenyl-propan-1-one, **11**, as the best intermediate from which to synthesize [$^2H_5$]-indinavir. Compound **11** was prepared from commercial [$^2H_5$]-bromobenzene.

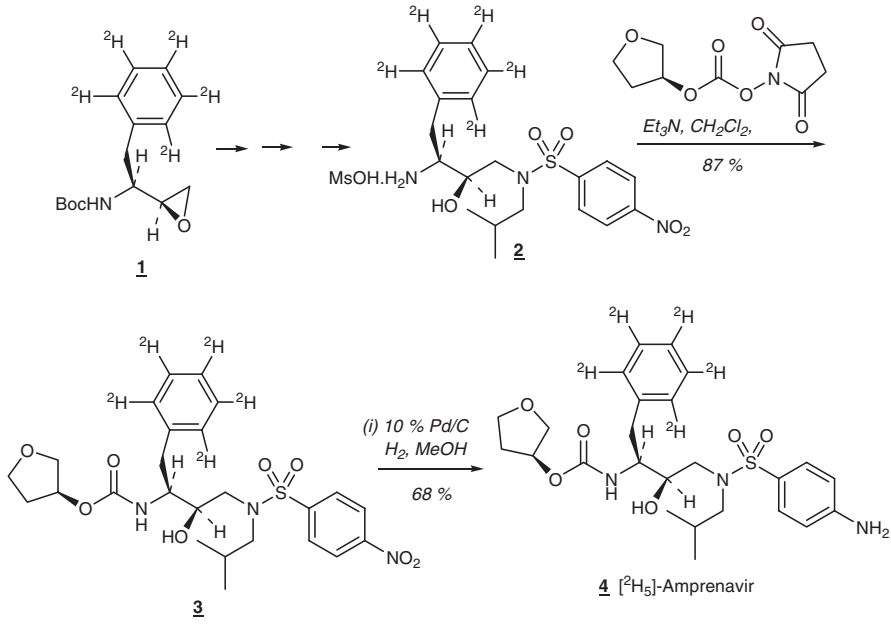
In this paper, we report the details of the sequence of reactions from compound **1**, which contains the [$^2H_5$]-HEA moiety, to [$^2H_5$]-amprenavir and [$^2H_5$]-saquinavir. We also report the use of compound **11** as a [$^2H_5$]-HEE precursor for the preparation of [$^2H_5$]-indinavir.

Results and discussion

The three-step sequence from (1S, 2S)-(1-oxiranyl-2-[$^2H_5$]phenylethyl)-carbamic acid *tert*-butyl ester, **1**, to 80% yield of *N*-(S)-3-amino-2R-hydroxyl-4-[$^2H_5$]phenylbutyl)-*N*-isobutyl-4-nitrobenzenesulfonamide, **2**, shown in Scheme 1, was described in an earlier paper.[5] It involved the reaction of isobutylamine with the epoxide **1**, formation of the 4-nitrobenzenesulfonamide derivative followed by the removal of the Boc group to yield the methanesulfonic acid salt, **2**.

[$^2H_5$]-AMPRENAVIR

From **2** only two further reaction steps were required to complete making [$^2H_5$]-amprenavir, **4**. These two steps involved the transformation of **2** to the (S)-3-hydroxytetrahydrofuran carbamoyl derivative **3** and the reduction of the nitro-group to the amine (see Scheme 1). We attempted to make compound **3** by two literature procedures.[6,7] The method whereby the chloroformate derivative of (S)-3-hydroxytetrahydrofuran is made by treating (S)-3-hydroxytetrahydrofuran with phosgene, followed by the addition of compound **2**, did not yield the desired carbamoyl compound **3**. The second approach, using (S)-3-tetrahydrofuranylsuccinimidyl carbonate, a reagent that other workers



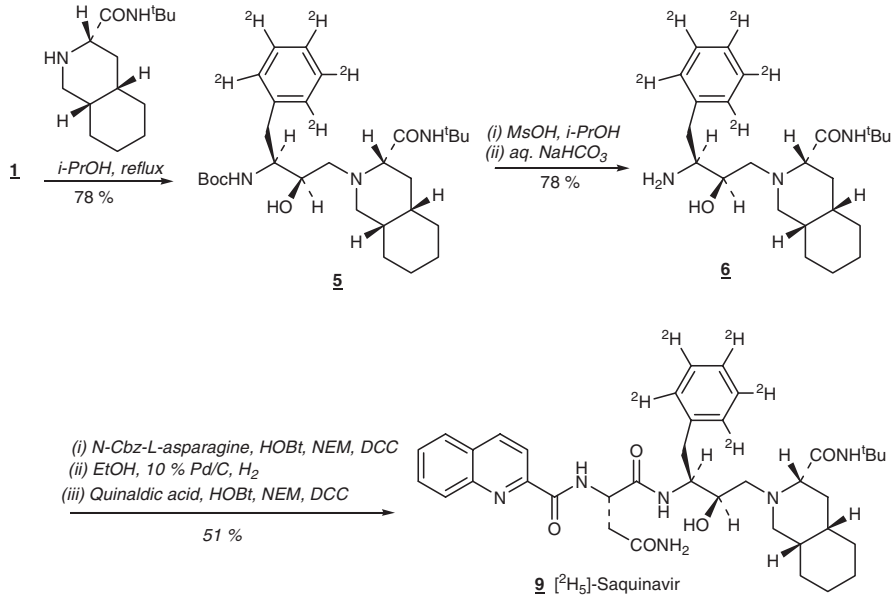
Scheme 1. Conversion of labeled epoxide 1 into amprenavir

have employed to convert amines to carbamoyl products in good yields,[7,8] was smoothly executed. We made the reagent, 3-(S)-tetrahydrofuranylsuccinimidyl carbonate, from 3-(S)-hydroxytetrahydrofuran and *N*,*N*-disuccinimidyl carbonate, according to the method of Ghosh.[8] Compound **2** was reacted with 3-(S-)-tetrahydrofuranylsuccinimidyl carbonate in the presence of triethylamine, and the reaction afforded **3** in 87% yield.

Catalytic hydrogenation of **3** in the presence of Pd/C gave a product which, after purification by flash chromatography on a Biotage silica gel column, afforded a 60% overall yield of [$^2H_5$]-amprenavir, **4**.

[$^2H_5$]-SAQUINAVIR

Prior to our study, the Hoffmann–La Roche group had reported the synthesis of saquinavir in several isotope labeled forms, including deuterium.[9] [$^2H_5$]-Saquinavir was made through the incorporation of penta-deuterated 2-quinolinoyl end-cap. Hexa-deuteroquinaldine was used in the two procedures, and one of the methods afforded 72.9 atom % [$^2H_5$] in the final product. The second method gave 63.3 atom % [$^2H_5$] product. Our earlier work[5] had indicated that insertion of the [$^2H_5$]-labeled HEA fragment with very high deuterium incorporation (>99 atom %) into PIs can be achieved using (1S, 2S)-(oxiranyl-2-[$^2H_5$]-phenylethyl)-carbamic acid *tert*-butyl ester, **1**. We have extended this approach to make [$^2H_5$]-saquinavir. Scheme 2 shows



Scheme 2. Conversion of labeled epoxide 1 into saquinavir

our sequence of reactions from (3S)-decahydroisoquinoline carboxamide and (1S, 2S)-(1-oxiranyl-2-[$^2H_5$]phenylethyl)-carbamic acid *tert*-butyl ester, **1**, to [$^2H_5$]-saquinavir. Compound **1** reacted with (3S)-decahydroisoquinoline carboxamide in refluxing isopropanol to provide **5**. The following reactions, which included removing the Boc group from **5**, gave compound **6** in 78% yield.

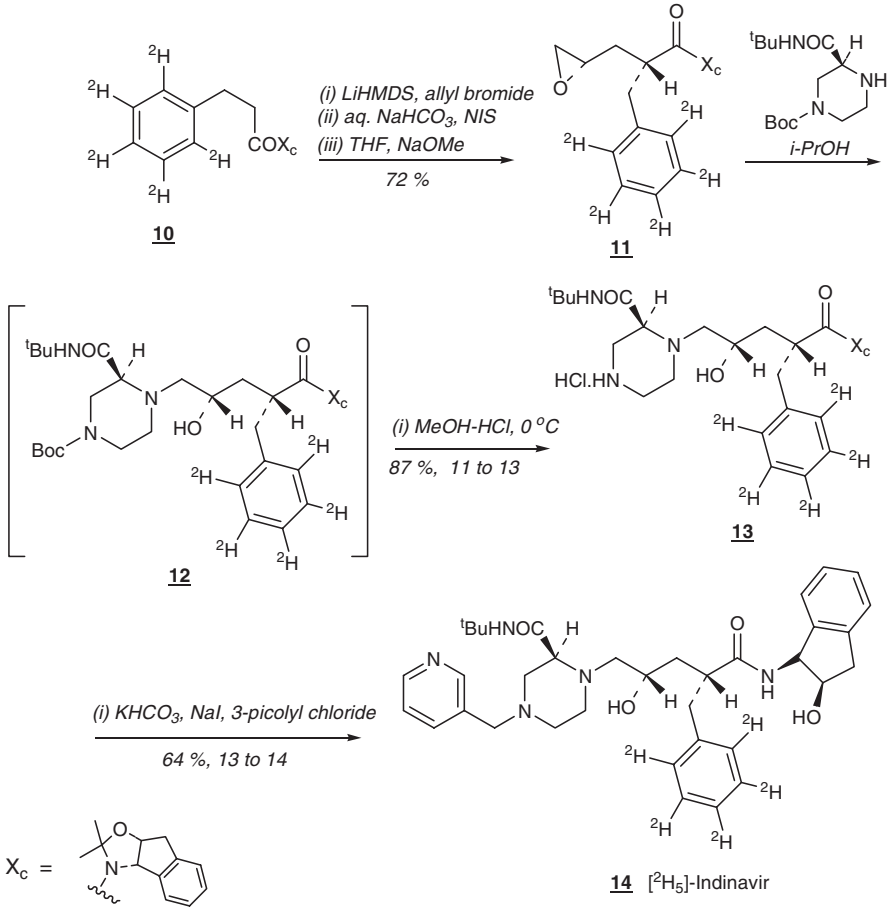
Subsequent steps involved the reaction of **6** with *N*-Cbz-L-asparagine to furnish {1-[1-[$^2H_5$]benzyl-3-(3-*tert*-butylcarbamoyl-octahydro-isoquinolin-2-yl)-2-hydroxyl-propylcarbamoyl]-2-carbomoyl-ethyl]-carbamic acid benzyl ester, Pd/C catalyzed hydrogenolysis of the Cbz group to provide 2-amino-*N*-[1-[$^2H_5$]benzyl-3-(3-*tert*-butylcarbamoyl-octahydro-isoquinolin-2-yl)-2-hydro-propyl]-succinamide, and coupling to quinaldic acid in the presence of HOBt and DCC, giving *N*-[1-[$^2H_5$]benzyl-3-(3-*tert*-butylcarbamoyl-octahydroisoquino-lin-2-yl)-2-hydroxy-propyl]-2-[(quinoline-2-carbamoyl)-amino]-succinamide, **9**. *N*-Ethylmorpholine was used as acid scavenger in the reaction forming ([$^2H_5$]-saquinavir) **9** in 73% overall yield. This sequence was modeled on an earlier literature synthesis of saquinavir.[10]

[$^2H_5$]-INDINAVIR

Since indinavir belongs to the 'HEE' transition state mimetic group of HIV-PIs, it required a different [$^2H_5$]-epoxide intermediate to make the [$^2H_5$]-labeled

product. 1S-(2,2-Dimethyl-8, 8a-dihydro-3aH-indeno-[1,2-d]-oxazol-3R-yl-2-oxiranylmethyl-3-[$^2H_5$]-phenylpropan-1-one, **11**, was selected as a starting structure for the phenyl-[$^2H_5$]-labeled HEE class of protease inhibitors, such as indinavir.

The chosen synthesis of [$^2H_5$]-indinavir is shown in Scheme 3. To start, [$^2H_5$]-hydrocinnamoyl chloride was prepared from commercially available [$^2H_5$]-bromobenzene. Compound **10** was made by the condensation of (1S,2R)-aminoindanol and [$^2H_5$]-hydrocinnamoyl chloride. The desired [$^2H_5$]-epoxide **11** was readily assembled from **10** by the use of a literature procedure,[11] involving sequential stereoselective allylation and epoxidation reactions. Opening the epoxide ring of **11** to provide **12** required the reagent *N*-*tert*-butyl-4-*N*-dimethylethylcarbamoyl-2S-piperazine carboxamide, which was made from 2-(S)-piperazine carboxylic acid according to a literature



Scheme 3. Preparation of labeled epoxide 11 and its conversion into indinavir

process.[12] Reaction of **11** with this reagent in isopropyl alcohol at 80°C furnished {4-[4-benzyl-5-(2,2-dimethyl)-8,8a-dihydro-3a*H*-indeno[1,2-o]oxazol-3-yl)-2-hydro-5-oxo-pentyl]-3-*tert*-butylcarbamoylpiperazin-1-yl}-carbamic *tert*-butyl ester, **12**. Compound **12** was de-blocked with a solution of hydrogen chloride in methanol to give **13** in 87% yield from **11**. The synthetic sequence was completed by the displacement reaction between **13** and 3-picolyl chloride in the presence of NaI and K_2CO_3, to give [$^2H_5$]-indinavir in 40% overall yield.

Salient spectral features

The insertion of a [$^2H_5$]-HEA subunit to make [$^2H_5$]-amprenavir leads to simplification of the proton NMR spectrum of the product. Figure 2 shows the aromatic regions of the proton NMR spectra of amprenavir and [$^2H_5$]-amprenavir. Three sets of resonances at δ 7.52 (d), 7.30–720 (m) and 6.66 (d), representing a total of nine protons in the ratio of (2:5:2), were observed for the two benzene ring systems in amprenavir. The multiplet

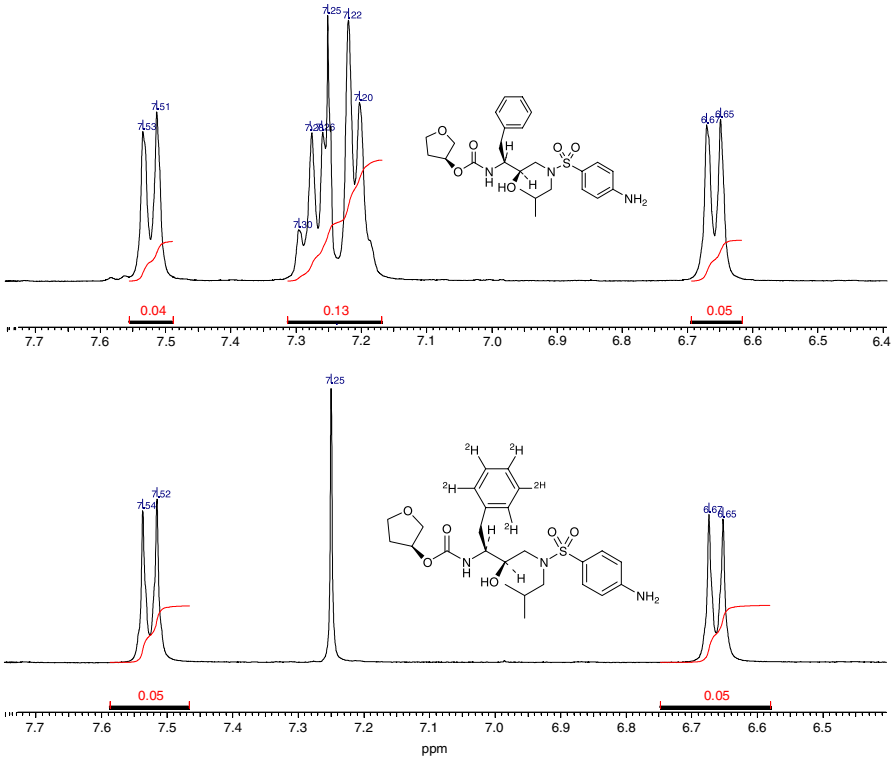


Figure 2. Aromatic region of ^{1}H-NMR spectra of amprenavir and [$^2H_5$]-amprenavir

at δ 7.30–7.20 (m) was assigned to the five protons in the phenyl substituent of the 'HEA' moiety of amprenavir. In [$^2H_5$]-amprenavir, where the [phenyl-$^2H_5$] moiety has been incorporated, the ^{1}H-NMR spectrum showed only two pairs of resonances at δ 7.52 and δ 6.66, for a total of 4 protons in the ratio of (1:1). This pattern is characteristic of protons in a para-substituted benzene ring system as in the 4-amino-benzenesulfonyl group. As would be expected, the multiplet at chemical shift δ 7.30–7.20 (m) due to the phenyl protons was absent in the proton NMR spectrum of the [phenyl-$^2H_5$]-amprenavir. Similar results were seen in [$^2H_5$]-saquinavir and [$^2H_5$]-indinavir, but were less obvious due to overlap of resonances of the additional aromatic groups present in each of those compounds.

Proton-decoupled ^{13}C-NMR spectra were similarly instructive as to the pattern of deuterium incorporation. The deuterium–carbon coupling constant (23.8 Hz) observable in the triplet resonance of a proton decoupled ^{13}C NMR spectrum was seen in [$^2H_5$]-amprenavir. Three triplet resonances were observed in the ratio of (2:2:1) with chemical shifts of 129.07, 128.23 and 126.02 ppm, respectively. Five additional single lines at 150.74, 137.45, 129.52, 126.26 and 114.11 ppm were attributable to the 4-amino-benzenesulfonyl-group, and these completed the expected number of aromatic carbons.

Mass spectrometry provided additional structural information. Figure 3 gives portions of the mass spectra of [$^2H_5$]-amprenavir, [$^2H_5$]-indinavir and [$^2H_5$]-saquinavir. All three compounds were determined to contain greater than 99 atom % [$^2H_5$]. Neither the [$^2H_2$] nor [$^2H_3$] species was detectable, and the [$^2H_4$] species was found to be present in less than 0.5% abundance. An alternate reported synthesis of [$^2H_5$]-saquinavir[9] indicated two different preparations of the material to consist of ($^2H_6$:$^2H_5$:$^2H_4$) in the ratio of 5.5:72.9:21.6 and ($^2H_6$:$^2H_5$:$^2H_4$:$^2H_3$) in the ratio of 2.8:63.3:27.3:6.6, respectively. In that earlier report, the loss or scrambling of deuterium during synthesis was suggested to lead to the variable composition of labeled species in the products.

Details from full scan ESI/MS(+) and MS/MS product ion spectra of m/z 506 from amprenavir and m/z 511 from [$^2H_5$]-amprenavir are given in the experimental section. A summary of ESI/MS/MS (+) and (−) CAD ion fragmentation are shown in Diagram 1. The fragmentation ions (m/z) from [$^2H_5$]-amprenavir are shown in parentheses. An examination of the fragmentation pattern of [$^2H_5$]-amprenavir relative to amprenavir clearly establishes the location of penta-deuteration as in the phenyl group of the 'HEA' isostere. This result also provides further confirmation for the absence of scrambling or loss of deuterium during synthesis.

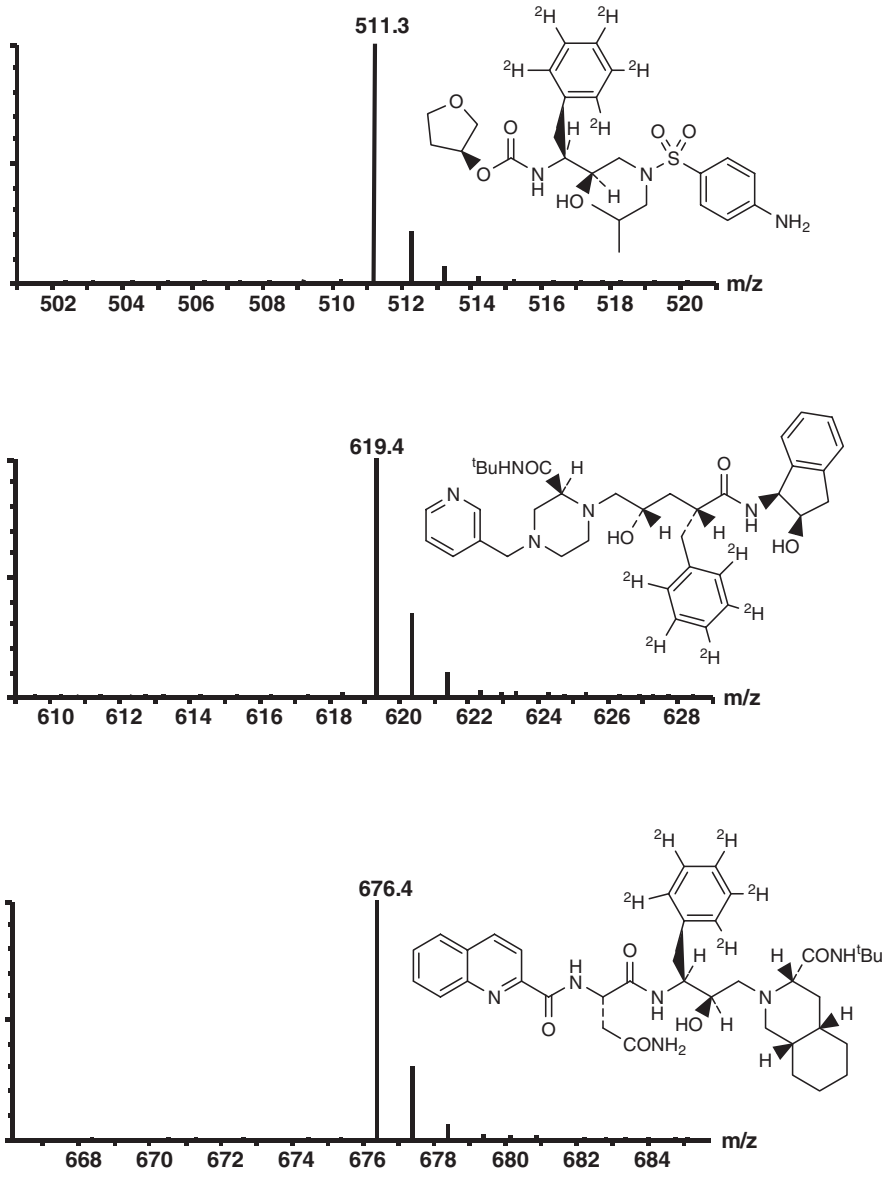


Figure 3. MS TOF ESI (+) of [$^2H_5$]-Pls

Conclusion

We have developed a sequence for synthesizing penta-deuterated forms of clinical HIV-protease inhibitors. By making [$^2H_5$]-amprenavir, [$^2H_5$]-saquinavir and [$^2H_5$]-indinavir, a potentially general route to [$^2H_5$]-HIV-PIs in both the HEA and HEE classes has been demonstrated. Labeled epoxide

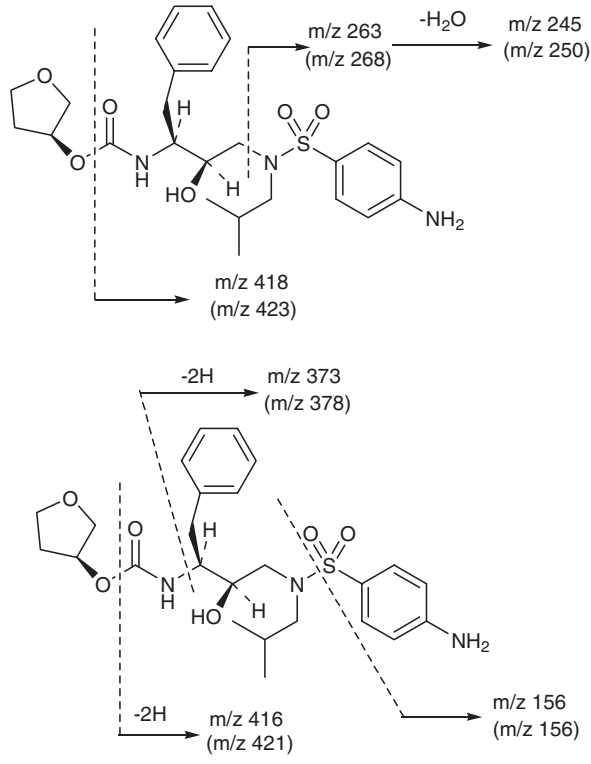


Diagram 1. ESI/MS/MS (+) CAD and ESI/MS/MS (−) CAD Ion Fragmentation of Amprenavir

intermediates were critical to the approach, and these compounds were readily made from commercially available [$^2H_5$]-bromobenzene. Very high deuterium incorporation is achievable by the approach we have described, and the absence of scrambling and/or loss of deuterium during synthesis is noteworthy. We believe this method of synthesis provides access to inexpensive, high quality [$^2H_5$]-labeled PIs for clinical research and mass spectrometry applications.

Experimental

All reactions were carried out under an atmosphere of argon unless otherwise specified. Solvents were commercial grade and used without purification or drying. Column chromatography was carried out on Merck Kieselgel 60 (230 μ) silica gel. Flash chromatographic separations were done using Biotage Flash System and pre-packed (90 g) silica gel cartridges. TLC visualization reagents included (10% iodine + 10% AcOH) in 40% aqueous KI. ^{1}H NMR spectra were recorded at 300, 400 or 500 MHz in $CDCl_3$. Chemical shifts are given in ppm relative to tetramethylsilane (TMS). ^{13}C-NMR spectra were

recorded at 100 MHz and chemical shifts given in ppm with $CDCl_3$ reference set at 77.0 ppm. Only the most important IR absorption (cm^{-1}) and the molecular ions and/or base peaks in mass spectra are given. HPLC analyses were completed using various columns, as specified in the experimental section. HRMS data were acquired on an LCT-TOF spectrometer (Micromass Ltd., Manchester, UK), in ESI mode, with a resolving power of $\sim$5000. Capillary voltage and sample voltage were at 3 and 25 kV. A desolvation temperature of 250°C and a source temperature of 120°C was used for all HRMS experiments.

{3S-[(4-Amino-benzenesulfonyl)-isobutylamino]-1S$^-$[$^2H_5$]-benzyl-2R-hydroxylpropyl]-carbamic acid tetrahydrofuran-3-yl ester ([$^2H_5$]-amprenavir) **4**

To a stirred solution of **2** (1.82 g, 3.5 mmol) and triethylamine (906 µl, 6.47 mmol) in dichloromethane (15 ml) was added 3-(S)-tetrahydrofuranyl-succinimidyl carbonate[8] (840 mg, 3.66 mmol) in dry dichloromethane (8 ml). After 3 h at room temperature, the reaction mixture was diluted with dichloromethane (30 ml) and washed successively with aqueous $NaHCO_3$ (2×20 ml), brine (30 ml) and dried on $MgSO_4$. The solvent was evaporated and a solution of the crude product in dichloromethane (10 ml) was applied to a column of silica gel. The compound was eluted with 30–45% ethyl acetate in hexane to afford **3** (856.0 mg, 87%). ^1H NMR (400 MHz, $CDCl_3$) δ 0.87 (dd, 6H), 1.59 (brs, 1H), 1.87 (m, 2H), 2.10 (m, 1H), 2.79 (m, 4H), 3.18 (m, 2H), 3.64 (d, 1H), 3.79 (m, 4H), 4.84 (d, 1H) 4.13 (brs), 7.94 (d, 2H), and 8.34 (d, 2H). Compound **3** was hydrogenated for 3 h at room temperature in methanol (60 ml) containing 10% Pd/C (250 mg) and filtered through a pad of Celite. The Celite was rinsed with methanol (30 ml), and the combined filtrate and washings was concentrated to a small volume. The material was applied to a column of silica gel and eluted with 2–5% ethanol in dichloromethane to give a solid. Crystallization of the solid from methanol yielded **4** [$^2H_5$]-amprenavir, (549 mg, 68%). HPLC on a Waters XTerra MS C_{18} 4.6×250 mm, 5 µ column eluted with the solvent combination of A (0.01 M ammonium acetate): B (acetonitrile); under gradient conditions: time 0–12 min, 40–60% B; time 12–18 min, 60–40% B; flow rate 1 ml/min, and peak detection at UV 254 nm, gave retention time of 8.74 min at greater than 99% chemical purity. MS acquired on an LCT-TOF spectrometer yield 511.4 $(M+H)^+$; [$^2H_5$] - 99.9 at%). ESI/MS(+) yielded molecular ion at m/z 511 $(M+H)^+$. MS/MS at CE 45 eV yielded ion m/z 423, and 250. The corresponding ESI/MS(+) for unlabeled amprenavir yielded m/z 506 $(M+H)^+$. MS/MS product ion at CE 45 eV produced m/z 418 and 245. ESI/MS(−) for [$^2H_5$]-amprenavir yielded m/z 569 $[M+Ac]^-$, 509, 421. MS/MS of m/z 421 yielded ions at 378 and 156. The corresponding ESI/MS(−) for unlabeled amprenavir gave 564 $[M+A]^-$, 504, 416. MS/MS of m/z 416 gave product ion at m/z 373 and

156. $[\alpha]_D^{20} + 8.22^\circ$ ($c = 0.49\%$ in MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (dd, 6H), 1.80 (m, 1H), 1.92 (m, 1H) 2.07 (m, 1H), 2.76 (dd, 1H), 2.95 (m, 3H), 3.12 (dd, 1H), 3.60 (d, 1H), 3.75 (dd), 3.83 (brs), 4.12 (m, 1H), 4.84 (d, 1H), 5.10 (brs), 6.67 (d, 2H) and 7.53 (d, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) 156.01, 150.74, 137.45, 129.52, 129.07 (tr), 128.23 (tr), 126.26, 126.02 (tr), 114.11, 75.30, 73.24, 72.55, 66.92, 58.83, 55.04, 53.76, 50.83, 35.34, 32.74, 27.30, 20.17, and 19.90.

[1-[$^2H_5$]-Benzyl-3-(3-tert-butylcarbamoyl-octahydro-isoquinolin-2-yl)-2-hydroxypropyl]-carbamic acid tert-butyl ester **5**

A mixture of (1S, 2S)-(oxiranyl-2-[$^2H_5$]-phenyl-ethyl)-carbamic acid *tert*-butyl ester **1** (2.5 g, 9.31 mmol) and (3S, 4aS, 8aS)-*N-tert*-butyl-decahydroisoquinoline-3-carboxamide (2.7 g, 11.32 mmol) in isopropyl alcohol (20 ml) was refluxed for 4 h and cooled to room temperature. The reaction mixture was concentrated to a residue and it was applied to a column of silica gel in a minimum volume of 2.5% EtOH in CH_2Cl_2. The column was first eluted with dichloromethane (400 ml) followed by 2.5% EtOH in CH_2Cl_2 to yield pure compound as a foamy solid **5** (3.70 g, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.88 (s, 1H), 4.83 (d, 1H), 3.85–3.58 (m, 3H), 3.02 (t, 1H), 2.90 (m, 2H), 2.65 (dd, 1H), 2.60 (d, 1H), 2.26 (m, 2H), 1.94 (q, 1H), 1.80–1.60 (m, 1H), 1.50–1.20 (m), 1.32 (s, 18H).

2-(3-Amino-2-hydro-4-[$^2H_5$]phenyl-butyl)-decahydro-isoquinoline-3-carboxylic acid tert-butylamide **6**

Methanesulfonic acid (521 µl, 8.03 mmol) in isopropyl acetate (5 ml) was added dropwise with stirring to 5 (3.60 g, 7.17 mmol) in isopropyl acetate (25 ml) maintained at 85°C. After 40 min, additional methanesulfonic acid (521 µl, 8.03 mmol) was added. TLC (silica gel, CH_2Cl_2:EtOH:NH_4OH; 90:10:0.25 v/v/v) after 1 h indicated the absence of the fast running starting compound. The mixture was cooled to room temperature, diluted with ethyl acetate (30 ml) and washed with a solution of $NaHCO_3$ (2 × 15 ml), brine (30 ml) and dried over magnesium sulfate. Solvent was evaporated and crude material was crystallized from methanol to afford **6** (2.27 g, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.18 (s, 1H), 3.65 (m, 1H), 3.20 (brs, 1H), 3.08 (m, 2H), 2.87 (dd, 1H), 2.70 (dd, 1H) 2.65 (dd, 1H), 2.47 (d, 1H), 2.33 (m, 2H), 1.87 (q, 1H), 1.79–1.60 (m, 3H), 1.56–1.20 (m), 1.33 (s, 9H).

N-[1-[$^2H_5$]Benzyl-3-(3-tert-butylcarbamoyl-octahydroisoquinolin-2-yl)-2-hydroxypropyl]-2-[(quinoline-2-carbamoyl)-amino]-succinamide ([$^2H_5$]-saquinavir) **9**

Cbz-L-Asparagine (498 mg, 1.86 mmol), HOBt (253 mg 1.86 mmol), 4-ethylmorpholine (237 µl, 1.86 mmol), dicyclohexylcarbodiimide DCC (424.9 mg, 2.05 mmol) in anhydrous THF (20 ml) was stirred under nitrogen atmosphere,

and **6** (760 mg, 1.86 mmol) was added. After 72 h the reaction mixture was concentrated to a paste and ethyl acetate (30 ml) was added. The mixture was washed with saturated NH_4Cl (2×25 ml), water (2×25 ml), saturated K_2CO_3 (30 ml), and finally brine (30 ml). The solution was evaporated on a rotary evaporator to give crude {1-[1-[$^2H_5$]benzyl-3-(3-*tert*-butylcarbamoyl-octahydro-isoquinolin-2-yl)-2-hydroxylpropylcarbamoyl]-2-carbamoylethyl]-carbamic acid benzyl ester. The material was dissolved in absolute ethanol (30 ml) and hydrogenated in the presence of 10% Pd/C (200 mg) at room temperature overnight. The reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated to a solid residue. A solution of the material in minimum dichloromethane was applied to a column of silica gel and the column was eluted with 3–5% ethanol in CH_2Cl_2 to afford 2-amino-*N*′-[1-[$^2H_5$]benzyl-3-(3-*tert*-butylcarbamoyl-octahydro-isoquinolin-2-yl)-2-hydropropyl]-succinamide (817.5 mg, 84%).

Quinaldic acid (218.3 mg, 1.26 mmol), 1-hydroxybenzotriazole, HOBt (170.3 mg, 1.26 mmol), dicyclohexylcarbodiimide, DCC (260.1 mg, 1.26 mmol), and *N*-ethyl-morpholine (NEM, 160.4 µl, 1.26 mmol) were added in succession to 2-amino-*N*′-[1-[$^2H_5$]benzyl-3-(3-*tert*-butylcarbamoyl-octahydro-isoquinolin-2-yl)-2-hydro-propyl]-succinamide (650 mg, 1.26 mmol) in dry THF (25 ml) at 5°C. After stirring for 20 h ethyl acetate (30 ml) was added and the reaction mixture filtered. The filtrate was washed with water (20 ml), 10% aqueous K_2CO_3 (20 ml), brine (30 ml) and dried over sodium sulfate. The solution was concentrated to a solid residue that was taken up in a minimum volume of ethyl acetate, applied to a column of silica gel, and eluted with 4–8% ethanol in CH_2Cl_2 to give a solid.

The recovered material was triturated with ether–petroleum ether to give *N*-[1-[$^2H_5$]benzyl-3-(3-*tert*-butylcarbamoyl-octahydroisoquinolin-2-yl)-2-hydroxypropyl]-2-[(quinoline-2-carbamoyl)-amino]-succinamide([$^2H_5$]-saquinavir, free base) **9** (520 mg, 61%). HPLC analysis was done with a Waters XTerra MS C_{18} 4.6×250 mm, 5 µ column. It was eluted with solvent combination of A (0.01 M ammonium acetate): B (acetonitrile) under gradient conditions: time 0–12 min, 40–60% B; time 12–18 min, 60–40% B at a flow rate of 1 ml/min, and the peak was detected by UV at 254 nm, giving a retention time of 12.7 min in >98% chemical purity. MS acquired on an LCT-TOF spectrometer gave 676.4 $(M+H)^+$; [$^2H_5$]-99.9 atom%). Optical rotation $[\alpha]_D^{20} - 50.32°$ ($c = 0.42\%$ in MeOH). MS gave 676 $(M+H)^+$ and others at m/z 338, 288 and 279. ^1H NMR (400 MHz, $CDCl_3$) δ 9.07 (d, 1H), 8.26 (d, 1H), 8.16 (d, 1H), 8.12 (d, 1H), 7.85 (d, 1H), 7.67 (tr, 1H), 7.61 (tr, 1H), 7.12 (brd, 1H), 6.13 (brd, 1H), 5.71 (brs, 1H), 4.82 (m, 1H), 4.24 (m, 1H), 3.89 (d, 1H), 3.03 (brd, 1H), 2.94–2.26 (m, 2H), 2.30 (brs, 1H), 1.95–1.6 (m, 5H), 1.38 (2H), and 1.29 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) 173.71, 173.04, 170.52, 164.60, 148.80, 146.52, 137.68, 137.35, 130.17, 130.03,

129.32, 128.79 (tr), 128.13, 127.79 (tr), 127.60, 125.60 (tr), 118.67, 70.71, 70.16, 59.13, 58.92, 54.52, 50.86, 50.25, 37.88, 35.75, 34.56, 33.20, 30.70, 30.65, 28.72, 26.13, 25.84, and 20.76.

1S-(2,2-Dimethyl-8,8a-dihydro-3aH-indeno[1,2-d]oxazol-3R-yl)-2-oxiranylmethyl-3-[$^2H_5$]-phenyl-propan-1-one **11**

Lithium bis(trimethysilyl)amide (LiHMDS) (29.6 ml, 29.6 mmol) was added slowly to **10** (8.65 g, 26.48 mmol) in anhydrous THF (120 ml) and stirred at -20°C under an argon atmosphere. After 1 h, allyl bromide (3.52 ml, 40.75 mmol) was added. The mixture was stirred for additional 1 h and quenched with saturated aqueous NH_4Cl solution (20 ml). The mixture was partitioned between water and ethyl acetate (300 ml, 1:2 v/v). The organic portion was separated and the aqueous phase was further extracted with ethyl acetate (2×120 ml). The organic portions were combined and washed with water (120 ml), brine (200 ml) and dried over magnesium sulfate. The crude material was applied as a solution in dichloromethane to a silica gel column and the compound was eluted with 10–20% EtOAc in hexane to give 2-[$^2H_5$]benzyl-1-(2,2-dimethyl-8,8a-dihydro-3aH-indeno[1,2-d]oxazol-3-yl)-pent-4-en-1-one (6.5 g, 67%). To a solution of the material (1.97 g, 5.34 mmol) in isopropyl acetate (30 ml) containing 2.0 M aq $NaHCO_3$ (5.40 ml, 10.8 mmol) was added *N*-iodosuccinimide (2.08 g, 9.24 mmol). After stirring the reaction mixture at room temperature for 1h, 10% aq sodium thiosulfate solution (8 ml) was added, and the organic phase was separated. The organic portion was further washed with water (3×15 ml), dried over anhydrous Na_2SO_4, and filtered. Sodium methoxide (0.5 M solution in THF, 21.5 ml, 10.68 mmol) was added to the filtrate. The reaction mixture was stirred for 30 min, and diluted with ethyl acetate (30 ml). It was washed with water (3×20 ml), brine (20 ml) and dried over anhydrous sodium sulfate. The solution was concentrated to a residue and the product was crystallized from ether–petroleum ether to give **11** (1.32 g, 64.4%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.16 (m, 2H), 6.88 (tr, 1H), 6.22 (d, 1H), 5.54 (d, 1H), 4.81 (tr, 1H), 3.46 (dd, 1H), 3.06 (m, 1H), 2.83 (m, 2H), 2.51 (m, 1H), 2.16 (m, 1H), 1.67 (s, 3H), and 1.43 (s, 3H).

1-[2-Hydoxy-4-(2-hydroxyindan-1-ylcarbamoyl)-5-[$^2H_5$]phenylpentyl]-piperazine-2-carboxyl acid tert-butylamide **13**

A mixture of **11** (800 mg, 2.09 mmol) and *N-tert*-butyl-4-*N*-dimethylethylcarbamoyl-2S-piperazine carboxamide (627 mg, 2.19 mmol) in isopropyl alcohol (20 ml) was heated at 80°C. After 6 h the reaction was cooled to room temperature and concentrated on a rotary evaporator to a residue. The material was applied to a column of silica gel as a solution in dichloromethane and eluted with 35% ethyl acetate in hexane to yield pure fractions. The

combined fractions were concentrated to a solid residue **12**, dissolved in dichloromethane (20 ml), and methanolic hydrogen chloride {prepared at 0–5°C under nitrogen atmosphere by addition of acetyl chloride (7 ml) to dry methanol (20 ml)} was added slowly with stirring at room temperature. After the Boc group was cleaved, as judged by TLC analyses (5% $EtOH/CH_2Cl_2$), the solvent was evaporated, and the residue was further azeotroped with toluene. The product was dissolved in a minimum volume of methanol, and diethyl ether was added slowly until the solid hydrochloride product separated. The precipitate was collected by filtration and dried under vacuum at 80°C to afford **13** (1.20 g, 87%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.29 (m, 1H), 7.19 (m, 3H), 5.22 (d, 1H), 4.354 (m 2H), 4.13 (tr, 1H), 4.02 (d, 1H), 3.74 (d, 1H), 3.59–3.38 (m, 4H), 3.13–2.99 (m, 5H), 2.87 (d, 1H), 2.77 (q, 1H), 1.90 (q, 1H), 1.52 (q, 1H), and 1.35 (s, 9H).

1-[2-Hydroxy-4-(2-hydroxy-indan-1-ylcarbamoyl)-5-[$^2H_5$]-phenylpentyl]-4-pyridin-3-ylmethyl-piperazine-2-carboxylic acid tert-butylamide, ([$^2H_5$]-indinavir) **14**

To a suspension of **13** (786 mg, 1.39 mmol) and K_2CO_3 (584 mg, 6.97 mmol) in dry THF/DMF (1:1 v/v, 30 ml) was added catalytic KI (≅10 mg) and 3-picolyl chloride (275 mg, 1.68 mmol). After stirring for 76 h at room temperature the mixture was diluted with ethyl acetate (60 ml) and transferred to a separatory funnel. The mixture was washed with water (2 × 40 ml), brine (50 ml), dried over magnesium sulfate and concentrated to a solid residue. The solid product was dissolved in 2% MeOH in CH_2Cl_2 and applied to a column of silica gel. The compound was eluted with 5–10% $EtOH/CH_2Cl_2$ containing 0.01% NH_4OH. The fractions containing pure product were combined and concentrated to a residue. The material crystallized from ethyl acetate to afford **14** [$^2H_5$]-indinavir (637 mg, 64%). HPLC analysis was completed using a Waters XTerra MS C_{18} 4.6 × 250 mm, 5 μ column. The column was eluted with a solvent combination of A (0.01 M Ammonium acetate): B (Acetonitrile); under gradient conditions: time 0–12 min, 40–60% B; time 12–18 min, 60–40% B; at a flow rate of 1 ml/min. The peak was detected by UV at 254 nm and gave a retention time of 7.0 min, with >97% chemical purity. MS acquired on an LCT-TOF spectrometer gave 619.4$(M+H)^+$; [$^2H_5$]-99.9 at%. Optical Rotation $[\alpha]_D^{20}$ + 8.96° ($c = 0.60\%$ in MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (m, 2H), 7.74 (brs, 1H), 7.59 (d, 1H), 7.28-7.7.10 (m), 5.91 (d, 1H), 5.26 (d, 1H), 4.25 (d, 1H), 3.79 (brs, 2H), 3.49 (brs, 2H), 3.16 (s, 1H), 3.03–2.49, 2.33 (m, 1H), 1.95 (m, 1H), 1.57 (m, 2H), 1.34 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) 175.06, 169.47, 150.49, 149.05, 140.58, 140.41, 139.79, 136.84, 132.51, 128.88 (tr), 128.14 (tr), 127.92, 126.76, 125.88 (tr), 125.16, 124.02, 123.44, 72.98, 65.86, 64.31, 61.50, 60.18, 57.47, 54.70, 52.68, 51.14, 48.02, 46.51, 39.54, 39.23, 38.12 and 29.03.

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References

1. (a) Kim EE, Baker CT, Dwyer MD, Murcko MA, Rao BG, Tung RD, Navia MA. *J Am Chem Soc* 1995; **117**: 1181–1182. (b) Tung RD, Murcko MA, Bhisetti GR, World Patent Appl 1994; March, publ. 17, No. WO94/05639.
2. Roberts NA, Martin JA, Kinchington D, Broadhurst AV, Craig JC, Duncan IB, Galphin SA, Handa BK, Krohn A, Lambert RW, Merrett JH, Mills JS, Parkes KBE, Redshaw S, Ritchie AJ, Taylor GJ, Machin PJ. *Science* 1990; **248**: 358.
3. (a) Vacca JP, Dorsey BD, Schleif WA, Levin RB, McDaniel SL, Darke PL, Zugay J, Quintero JC, Blahy OM, Roth E, Sardana VV, Schlabach AJ, Graham PI, Condra JH, Gotlib L, Halloway MK, Lin J, Chen I-W, Vastag K, Ostovic D, Anderson PS, Emini EA, Huff R. *Proc Natl Acad Sci USA* 1994; **91**: 4096–4100. (b) *J Med Chem* 1994; **37**: 3443–3451.
4. Kaltenbach III RF, Trainor D, Getman G, Harris S, Garber B, Cordova L, Bacheler S, Jeffery K, Logue P, Cawood R, Klabe S, Diamond M, Saye JJ, Erickson-Viitanen S. *Antimicrob Agents Chemother* 2001; **45**: 3021–3028.
5. Ekhato IV, Liao Y, Plesescu M. *J Label Compd Radiopharm* 2004; **47**: 821–835.
6. Beaulieu PL, Wernic D. *J Org Chem* 1996; **61**: 3635–3645.
7. Kim BM, Bae SJ, So SM, Yoo HY, Chang SK, Lee JH, Kang J. *Org Lett* 2001; **3**: 2349–2351.
8. Ghosh AK, Duong TT, Mckee SP, Thompson WJ. *Tertrahedron Lett* 1992; **33**: 2781–2784.
9. Wiltshire HR, Prior KJ, Dhesi J, Trach F, Schlageter M, Schonenberger H. *J Label Compd Radiopharm* 1998; **41**: 1103–1126.
10. Parkes KEB, Bushnell DJ, Crackett PH, Dunsdon SJ, Freeman AC, Gunn MP, Hopkins RA, Lambert RW, Martin JA, Merrett JH, Redshaw S, Spurden WC, Thomas GJ. *J Org Chem* 1994; **59**: 3656–3664.
11. Maligres PE, Upadhyay V, Rossen K, Cianciosi SJ, Purick RM, Eng KK, Reamer RA, Askin D, Volante RP, Reider PJ. *Tetrahedron Lett* 1995; **36**: 2195–2198.
12. (a) Dorsey BD, Levin RB, McDaniel SL, Vacca JP, Guare JP, Darke PK, Zugay JA, Emini EA, Schleif WA, Quintero JC, Lin JH, Chen I-W, Holloway MK, Fitzgerald PMD, Axel MG, Ostovic D, Anderson PS, Huff JR. *J Med Chem* 1994; **37**: 3443–3445. (b) Askin D. *Curr Opinion Drug Discov Develop* 1998; **1**: 338–348 and reference cited therein.

